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Seroprevalence and risk factors associated with maedi visna virus in sheep population in southwestern Croatia

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ABSTRACT

The objective of this cross-sectional study was to detect the presence of maedi visna virus (MVV) infections in sheep in three counties in southwestern Croatia: Istria County, Primorje-Gorski Kotar County and Lika-Senj County, and to estimate the influence of geographical location, breed, age and gender as risk factors on the prevalence rate. A total of 460 randomly selected sheep were tested using a commercially available ID Screen® MVV/CAEV Indirect screening test, VISNAS-5P (ID.VET, Grabels France) for detection of antibodies against the Maedi-Visna virus. The prevalence rate for the entire population was 10.00 % (95 % 7.26 - 12.74 %), The highest seroprevalence of MVV was found in Istria County (19.02%, CI 95% 13.35 –24.70%), followed by Lika-Senj County (5.43 %, CI 95 % 2.17- 8.70) and Primorje-Gorski Kotar County (1.07 %, CI 95 % -1.03 – 3.17) (P<0.01). When comparing the seroprevalence among breeds, statistically significant differences were observed. The largest number of positive sheep was found in the following breeds: Istrian Pramenka (22.97%, CI 95% 13.39 - 32.55%), Jezersko-Solcava sheep (23.53%, CI 95% 11.89 - 35.17%%), Pramenka (13.16%, CI 95% 2.41 - 23.91%) and Lika sheep (9.78%, CI 95% 3.71- 15.85%). Prevalence rates in other examined breeds (Krk sheep, Pag sheep and Romanov sheep) were lower than 2 % (P<0.01). No statistically significant differences in seroprevalence between sex and age were found. According to risk estimation, the main risk factors were found to be geographical location and breed. In Istria County, seroprevalence was 4.77 (CI 95 % 2.67 – 8.54) higher than in other examined areas (OR = 5.66, CI 95 % 2.97-10.79) (P<0.01). As for breeds, significant differences were observed between Jezersko-Solcava sheep and Lika sheep (OR = 2.84, CI 95% 1.13-7.14) and Istrian Pramenka in relation to Pag sheep (OR 22.51,, CI 95% 16.99-119.73), Krk sheep (OR 19.53, CI 95% 5.80-108.27), and Lika sheep (OR 2.35, CI 95% 1.17-6.47). This cross-section study indicates the circulation of MVV in this sheep population in Croatia, and represents the first research into the risk factors for MVV in sheep in southwestern Croatia.

Key words: Maedi-Visna virus; sheep; seroprevalence; risk factors

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Introduction

Maedi-visna (MV) is a chronic viral disease of sheep caused by the maedi/visna virus (MVV), characterized by a long incubation period and slow progression (HOUWERS and VAN DER MOLEN, 1987; NARAYAN and CLEMENTS, 1989). Clinical signs are usually determined as interstitial pneumonia (Maedi) and progressive inflammatory encephalitis (Visna), as well as arthritis and mastitis. Clinical signs develop slowly and can differ between breeds. About 30 % of infected animals do not develop clinical signs. Infection is lifelong and persistent (VAN DER MOLEN and HOUWERS, 1987; CUTLIP et al., 1988; HOUWERS et al., 1988; NARAYAN and CLEMENTS, 1989; PEPIN et al., 1998; BOLEA et al., 2006; BENAVIDES et al., 2007; GREGORY et al., 2009; AKKOC et al., 2011; KALOGIANNI et al., 2020). MVV with caprine arthritis-encephalitis virus (CAEV), commonly known as small ruminant lentiviruses (SRLV), belongs to the genus Lentavirus and to the family Retroviridae (BLACKLAWS, 2012; RAMIREZ et al., 2013; MINGUIJON et al., 2015).

MVV is mainly transmitted by the respiratory route through aerosols and colostrum (PETERHANS et al., 2004; SHAH et al. 2004), and has a tropism for monocyte/macrophages and lymphoproliferative lesions located mainly in the lungs, the central nervous system, udder and joints (NARAYAN et al., 1982; PREZIUSO et al., 2009; 2013). MV is a disease that causes huge economic losses in sheep breeding associated with reduced production and increased replacement rates due to chronic mastitis, pneumonia, arthritis and early culling (SNOWDER et al., 1990; PETERHANS et al., 2004).

The prevalence rate in European countries is observed as negative (MVV free), very low (1–9 %), low (10–39 %), intermediate (40–69 %), and high (> 70 %) (REINA et al., 2009). A very low prevalence rate was observed in Finland (SIHVONEN et al., 1999), Poland (JUNKUSZEWA et al., 2016), Belgium (MICHIELS et al., 2018) and Switzerland (SCHALLER et al., 2000) while a low prevalence rate was found in Croatia (VIDIĆ et al., 2017), Turkey (AZKUR et al., 2011; AMEEN and KARAPINAR, 2018) and Spain (LAGO et al., 2012). An intermediate prevalence rate was also observed in Spain (PEREZ at al., 2010), Greece (KARANIKOLAOU et al., 2005), North Macedonia (MITROV et al., 2009) and Kosovo (CANA et al., 2020). MVV infection has been recorded ranging from 4.60 to 50.00 % in China (ZHANG et al., 2013), Japan (OGUMA et al., 2014) and Iran (BEHNAZ et al., 2015).

The prevalence rate depends on risk factors. The production system, including housing and the management system, has a major impact on seroprevalence (PETERHANS et al., 2004; LEGINAGOIKOA et al., 2010). Higher prevalence rates have been observed in developed countries and in intensively reared sheep than in semi-intensively and extensively reared sheep (LEGINAGOIKOA et al., 2006; 2010, REINA et al., 2009; BARQUERO et al., 2013; KALOGIANNI et al., 2020). However, it seems that environmental factors related to geographical location are also of great importance as risk factors (BARQUERO, et al., 2013; BEHNAZ et al., 2015; BOJAR et al., 2018.) as well as intrinsic factors, such as breed (CUTLIP et al., 1988; KEEN et al., 1997; BARQUERO et al., 2013), age (PREZIUSO et al., 2010.; BEHNAZ et al., 2015; CANA et al., 2020) and gender (KEEN, 1997; PREZIUSO et al., 2010).

The increase in prevalence rate and huge economic losses in sheep breeding have led to the need for the development of control programmes in Europe. A European Co-operation in the field of Scientific and Technical Research (COST) campaign was initiated in 1998 to coordinate research in 16 European countries, and to issue recommendations on control policies. According to these recommendations, all European countries should determine the prevalence of lentiviruses by survey (PETERHANS et al., 2004).

In the Republic of Croatia, sheep breeding has a long tradition, as well as sociological, cultural and ethnological roots. According to the Annual Report on the system of sheep and goat identification and registration in the Republic of Croatia for 2018 (BARAĆ et al., 2019) and the Unique Register of sheep and goats, about 580,000 sheep were raised in Croatia in 2018. Breeding work was performed with a population of 41,387 breeding sheep (7.14%

of the total sheep population) on 396 farms. In some regions, sheep breeding has promising economic potential for Croatia's livestock and agriculture in general. On the other hand, seroprevalence of MVV in sheep in Croatia has been poorly studied. VIDIĆ et al. (2017) reported on the seroplevalence of MVV in Dalmatia, where the prevalence rate was 16.18% at animal level and 15.9% at flock level. However, the prevalence rate of MVV infection in the sheep population in other regions of Croatia is not known. Therefore, the objective of this cross-sectional study was to detect the presence of MVV infection in sheep, and to determine the level of seroprevalence in the southwestern part of Croatia, as well as to assess risk factors.

Material and methods

Study area and sample collection. A total of 460 serum samples were obtained from randomly selected sheep of different ages and both sexes, belonging to 17 breeders (flocks) located in three counties in the southwestern part of Croatia: Istria County (N=184), Primorsko-Goranska County (N=92) and Lika-Senj County (N=184). Blood samples were collected from April to July 2019 and delivered to the Croatian Veterinary Institute, Veterinary Department, Rijeka. The blood samples were centrifuged at 2000 RPM for 10 minutes and the serum samples were stored at - 20 °C until being tested. All sera samples were tested for antibodies against maedi-visna virus by commercial ID Screen® MVV/CAEV Indirect screening test VISNAS-5P (ID.VET, Grabels, France). Tests were carried out according to the manufacturer's instructions.

Statistical and risk factor analysis. Statistical analysis included descriptive statistics, frequency distributions, distribution fitting, Student-t test and nonparametric tests, the Mann-Whitneyev test when two groups were tested, and Kruskal-Wallis analysis for multiple analysis. For binary and categorical variables, statistically significant differences in seroprevalence among groups were assessed using the chi-square statistics when more than two groups were involved, and the Fisher exact test for 2-way comparisons only. The continuous variables, such as age, were evaluated using t-statistics. All sample seroprevalence was estimated for the entire population calculating the standard error (SE) and 95% confidence limit (CI 95 %). In order to evaluate the strength of association between MVV infection and the risk factors, including geographical location (counties), breed, age and gender, the prevalence ratio (PR) as well as odds ratio (OR) and the corresponding 95 % confidence intervals and P-values were calculated. To provide valid estimates of the strength of association among the groups, logistic regression analysis was conducted (HOSMER and LEMESHOW, 2000). The significance between groups was taken at the level of 5 % ($p < 0.05$) for a double-sided test. All statistical analyses were performed using SATATISTCA 12 software and WinEpiscope ver. 2.

Results

Out of 460 examined sheep, 46 (10.00%, CI 95% 7.26 –12.74%) were positive, while in l17 examined herds, the seroprevalence at herd level was 58.82% (CI 95%, 35.42 - 82.22%). The number of examined and positive sheep by counties is shown in Table 1.

Table 1. Seroprevalence of maedi-visna virus in sheep and herds by geographical location (counties)

County	Sheep examined				Flock examined				SE for sample	SE for herd
	N	Positive			N	Positive				
		N	%	CI 95 %		N	%	CI 95 %		
Istria	184	35	19.02	13.35 -24.70	8	6	75.00	44.99 – 105.01	0.029	0.15
Primorje-Gorski Kotar	92	1	1.07	–1.03 –3.17	4	1	25.00	-17.44 – 67.43	0.011	0.21
Lika-Senj	184	10	5.43	2.17 – 8.70	5	3	60.00	17.06–102.94	0,017	0.22
Total	460	46	10.00	7.26 – 12.74	17	10	58.82	35.42 – 82.22	0.014	0.12

SE standard error

As shown in Table 1, the highest seroprevalence of MVV in sheep was found in Istria County (19.02 %, CI 95 % 13.35 –24.70 %), then Lika-Senj County (5.43 %, CI 95 % 2.17- 8.70) and Primorje-Gorski Kotar County (1.07 %, CI 95 % -1.03 – 3.17) (P<0.01). The highest seroprevalence at herd level was also found in Istria County (75.00 %, CI 95 % 44.99 –105.01 %) followed by Primorje-Gorski Kotar County (60.00 %, CI 95 % 17.06 - 102.94 %) while the lowest prevalence rate was observed in Lika-Senj County (25.00 %, CI 95 % -17.44 –67.43). These differences were not found to be statistically significant (P>0.05).

The seroprevalence of MVV infection in sheep by breed, gender and age is shown in Table 2.

Table 2. Seroprevalence of maedi-visna virus infection in sheep in relation to breed, gender and age

Breed	Sheep examined	Sheep positive		CI 95%		SE
	N	N	%	Lower	Upper	
Pag sheep	96	1	1.04	-0.98	3.07	0.010
Lika sheep	92	9	9.78	3.71	15.85	0.031
Krk sheep	85	1	1.18	-1.12	3.48	0.012
Istrian Pramenka	74	17	22.97	13.39	32.55	0.049
Jezersko-Solcava sheep	51	12	23.53	11.89	35.17	0.059
Pramenka	38	5	13.16	2.41	23.91	0.054
Romanov sheep	12	0	0	0	0	0
East Friesian sheep	3	1	33.33	-20.01	86.67	0.272
Not specified	9	0	0	0	0	0
Gender						
Female	436	45	10.32	7.47	13.17	0.014
Male	14	1	7.14	-6.34	20.63	0.069
Not specified	10	0	0	0	0	0
Age (years)						
0-2.0	29	0	0	0	0	0
2.1-6.0	161	17	10.56	5.81	15.31	0.024
6.1-10	139	16	11.51	6.20	16.82	0.027
10.1 and more	67	9	13.43	5.27	21.59	0.043
Not specified	64	4	6.25	0.32	12.18	0.030
Total	460	46	10.00	7.26	12.74	0.014

SE standard error

Comparing the seroprevalence between breeds, the highest seroprevalence was found in Istrian Pramenka (22.97 %, CI 95 % 13.39 - 32.55 %) and Jezersko-Solcava sheep (23.53 %, CI 95 % 11.89 - 35.17 %) followed by Pramenka (13.16 %, CI 95 % 2.41 - 23.91 %) and Lika sheep (9.78 %, CI 95 % 3.71 - 15.85 %). Differences between these four breeds were not statistically significant (P>0.05), while when comparing the prevalence rate observed in Krk sheep, Pag sheep and Romanov sheep, significant differences were found (P<0.05) (Table 2).

The largest number of examined sheep were female (N=436) out of which 10.32% (CI 95 % 7.47 - 13.17 %) were positive, while out of the 14 male sheep examined, only one animal was positive (7.14%, CI 95 % 6.34 - 20.63 %). The difference between genders was not statistically significant ($\chi 2$ = 0.149, P=0.6990). The positive male belonged to the Istrian sheep breed from Istria County.

To estimate the seroprevalence of sheep by age, four age groups (Table 2) were created according to the distribution fitting and the mean age (Table 3). For the age groups approximately the range of ± 1 standard deviation was taken (Table 2). There were no positive individuals up to two years of age. No significant differences between age groups were found (P>0.05).

Tables 3 to 5 show the basic statistical parameters for age in relation to breed, gender and positive and negative results.

Table 3. Basic statistical parameters for age in relation to breed

Breed	N	Mean	Median	Mode	Frequency of mode	Min	Max	Lower quartile	Upper quartile	SD	SE	Skewness	Kurtosis
Pag sheep	85	7.26*	6.30	6.30	14	2.10	14.30	4.30	9.30	3.49	0.38	0.55	-0.81
Lika Pramenka	85	5.26	5.30	1.00	9	1.00	14.30	2.30	7.30	3.29	0.38	0.64	-0.20
Krtk sheep	49	6.41*	6.10	2.30	9	1.10	18.00	2.30	8.00	4.44	0.62	0.98	0.33
Istrian Pramenka	74	5.50	5.05	Multiple	5	0.11	10.40	3.60	7.70	2.75	0.32	-0.01	-1.04
Jezersko-Solcava sheep	51	7.67*	7.90	Multiple	5	1.80	13.40	2.70	12.30	4.04	0.86	0.60	-0.78
Pramenka	38	6.70*	6.25	Multiple	4	2.50	14.30	4.11	9.40	3.10	0.51	0.69	-0.48
Romanov sheep	11	3.98*	3.11	2.10	4	2.10	13.40	2.10	5.10	3.32	1.00	2.68	7.75
East Friesian sheep	3	8.37	8.40	Multiple	1	6.30	10.40	6.30	10.40	2.05	1.18	-0.07	
Total	396	6.28	6.20	6.30	28	0.11	18.00	3.30	8.40	3.59	0.18	0.61	-0.27

*P<0.05

Table 4. Basic statistical parameters for age in relation to gender

	N	Mean	Median	Mode	Frequency of mode	Min	Max	Lower quartile	Upper quartile	SD	SE	Skewness	Kurtosis
Female	383	6.41*	6.30	6.30	26	1.00	18.00	3.30	8.60	3.57	0.18	0.59	-0.29
Male	13	2.47*	2.40	1.00	3	0.11	5.30	1.00	3.60	1.59	0.44	0.31	-1.00
Total	396	6.28	6.20	6.30	28	0.11	18.00	3.30	8.40	3.59	0.18	0.61	-0.27

*P<0.01

Table. 5 Basic statistical parameters for age in relation to positive and negative serological tests

	N	Mean	Median	Mode	Frequency of mode	Min	Max	Lower quartile	Upper quartile	SD	SE	Skewness	Kurtosis
Negative animals	354	6.15*	6.10	6.30	27	0.11	18.00	3.20	8.30	3.62	0.19	0.66	-0.16
Positive animals	42	7.38*	7.35	Multiple	4	2,60	13.40	4.10	9.30	3.18	0.49	0.39	-1.08
Total	396	6.28	6.20	6.30	28	0.11	18.00	3.30	8.40	3.59	0.18	0.61	-0.27

*P<0.05

Out of the total number of examined sheep (N=460), age was known for 396 sheep. The mean of age in the sheep was 6.28 ± 3.59, ranging from a minimum of 0.11 years to a maximum of 18 years. Statistical differences in age were found between breeds (P<0.05). The significantly older sheep were Pag sheep and Jezersko-Solcava sheep breeds, followed by Krk sheep and Pramenka, while Romanov sheep were significantly younger than the other breeds (Table 3).

Out of the total females examined (N=436), age was known for 383 ewes, while out of 14 male

sheep examined, age was determined in 13 rams. The average age of the ewes was 6.30 ± 3.57 years, ranging from 1 to 18 years, while the average age of the rams was 2.47 ± 1.59 years, ranging from 0.11 to 5.30 years (t-statistic -3.962., P=0.0001) (Table 4).

The average age of seropositive and seronegative animals was 7.38 ± 3.18 and 6.15 ± 3.62, respectively. The difference was statistically significant (t-statistic 2.107, P=0.0357) (Table 5).

Table 6 shows the seroprevalence of MVV in sheep in relation to breed, gender and age, by counties.

Table 6. Seroprevalence of MVV in sheep in relation to breed, gender and age by counties

County	Sample examined	Positive			SE
		N	%	CI 95 %	
Istria	184	35	19.02	13.35 –24.91	0.029
Breed					
Istrian Pramenka	74	17	22.97	13.39 –32.55	0.049
Jezersko- Solcava sheep	51	12	23.53	11.89 –35.17	0.059
Pramenka	38	5	13.16	2.45 –23.91	0.055
Romanov sheep	12	0	0	0	0
East Friesian sheep	3	1	33.33	-20.01 –86.67	0.273
Not specified	6	0	0	0	0
Gender					
Male	9	1	11.11	-9.42 –31.64	0.105
Female	175	34	19.43	13.57 –25.29	0.029
Not specified	-	-	-	-	-
Age (years)					
0-2	14	0	0	0	0
2.1-6.0	72	13	18.06	9.17 – 26.95	0.045
6.1-10	62	14	22.58	12.17 – 32.99	0.053
10.1 and more	27	8	29.63	12.41 – 46.85	0.088
Not specified	9	0	0	0	0
Primorje-Gorski Kotar	92	1	1.09	-1.03 –3.21	0.011
Breed					
Krk sheep	85	1	1.18	-1.11 – 3.48	0.011
Pag sheep	4	0	0	0	0
Not specified	3	0	0	0	0
Gender					
Male	0	-	-	-	-
Female	89	1	1.12	-1.07 –3.31	0.112
Not specified	3	0	0	0	0
Age (years)					
0-2.0	0	0	-	-	-
2.1-6.0	19	0	0	0	0
6.1-10	25	0	0	0	0
10.1 and more	11	0	0	0	0
Not specified	37	1	2.70	-2.52 – 7.92	0.027
Lika Senj	184	10	5.43	2.16 – 8.70	0,017
Breed					
Pag sheep	92	1	1.09	-1.03 – 3.21	0.011
Lika sheep	92	9	9.78	3.71 – 15.85	0.031

Table 6. Seroprevalence of MVV in sheep in relation to breed, gender and age by counties (continued)

County	Sample examined	Positive			SE
		N	%	CI 95 %	
Gender					
Male	5	0	0	0	0
Female	172	10	5.81	2.31 – 9.31	0.018
Not specified	7	0	0	0	0
Age (years)					
0-2.0	11	0	0	0	0
2.1-6.0	69	4	5.80	0.28 – 11.32	0.028
6.1-10	57	2	3.51	-1.27 – 8.29	0.024
10.1 and more	29	1	3.45	-3.19 – 10.09	0.034
Not specified	18	3	16.67	-4.98 – 11.88	0.043

When comparing the prevalence rate among the breeds by counties, it can be seen, as presented in Table 6, that the largest number of seropositive sheep was found in Istria County in the domestic breeds Istrian Pramenka (22.97, CI % 13.39 – 32.55%) and Pramenka (13.16, CI% 2.45 – 23.91%), as well as in the not-domestic breed, Jezersko-Solcava sheep (25.53, CI % 11.89 – 35.17%). These differences were not found to be statistically significant (P>0.05). Statistical significance was observed between the domestic breeds Lika sheep (9.78%, CI% 3.71 – 15.85%) and Pag sheep (1.09 %, CI 95% -1.03 – 3.21 %) in Lika-Senj County (P<0.01). Pag sheep in both counties (Primorje–Gorski Kotar County and Lika-Senj County) showed very low seropositivity.

In all counties, seroprevalence was higher in ewes than in rams. Comparing the prevalence of female sheep between the counties, there were significantly more (P<0.01) positive ewes in Istria County (19.43. CI 95 % 13.57 - 25.29 %) compared to Primorje-Gorski Kotar and Lika-Senj Counties, with a prevalence of 1.12% (CI 95 % 1.11 - 3.48 %) and 5.81 % (CI 95 % 2.31 - 9.31 %), respectively.

The results of logistic regression analysis are summarised in Table 7.

Table 7. Results of logistic regression analysis for variables related to the risk factors associated with the MVV seroprevalence in sheep

Risk factor	Variables	OR[1]	CI 95 % [2]	P-value [3]
Geographical location	Primorje-Gorski Kotar	reference		
	Istrian County	21.38	2.88 – 158.71	0.0028
	Lika Senj County	4.06	1.95 – 8.49	0.0002
Breed	Pag sheep	reference		
	Krk sheep	1.12	0.07 – 18. 15	0.0780
	Lika sheep	10.30	1.28 – 83.03	0.0285
	Pramenka	14.39	1.62 – 127.75	0.0167
	Istrian Pramenka	28.33	3.67 – 218.64	0.0013
	Jezersko-Solcava sheep	29.23	3.67 – 232.63	0.0014
Breed according to geographical location	Istrian County			
	Pramenka	reference		
	Istrian Pramenka	1.99	0.66 – 5.83	0.2214
	Jezersko-Solcava sheep	2.03	0.65 – 6.36	0.0039
	Primorje-Gorski Kotar			
	Pag sheep	reference		
	Krk sheep	0.16	0.001 . 4.50	0.2815
	Lika-Senj County			
	Pag sheep	reference		
	Lika sheep	9.87	1.22 – 79.56	0.0316

Table 7. Results of logistic regression analysis for variables related to the risk factors associated with the MVV seroprevalence in sheep (continued)

Risk factor	Variables	OR[1]	CI 95 % [2]	P-value [3]
Gender	Male	reference	0.19 – 11. 71	
	Female	1.50		0.7011
Age (years)	2 – 6	reference		
	6 – 10	1.11	0.53 – 2.28	0.7928
	more than 10	1.31	0.55 – 3.17	0.5349

[1] odds ratio, [2] confidence interval for OR, [3] significance P<0.05

The analysis of risk factors for MVV showed that in Istria County it is 21.38 (CI 95 % 2.88 – 158.71) times and in Lika-Senj County 4.06 (CI 95 % 1.95 – 8.49) times more probable that sheep will be positive than in the Primorje-Gorski Kotar County (P<0.01). In relation to breed, the likelihood for infection is much higher in Jezersko-Solcava, Istrian Pramenka and Pramenka sheep than in Lika sheep, Pag sheep and Krk sheep (OR = 6.28, CI 95% 3.08 – 12.79) (P<0.0001). The association between seropositivity and gender, as well as age, was not found to be statistically significant (P>0.05).

Discussion

The serological survey of maedi-visna virus (MVV) infection in southwestern Croatia shows the presence of MVV infection in the study population. Out of the total study population in three geographical locations (Istria County, Primorje-Gorski Kotar County and Lika-Senj County), 10 % (CI 95 % 7.26 –12.74 %) of tested animals were seropositive. Out of 17 study flocks, 58.82 % (CI 95%, 35.42 - 82.22%) were positive. On the other hand, in a population in south Croatia (Dalmatia), including four counties (Dubrovnik-Neretva, Split-Dalmatia, Šibenik-Knin and Zadar County) VIDIĆ et al (2017) found a seroprevalence of 16.18 %. Different prevalence rates, ranging from very low to very high have been recorded worldwide. Seroprevalence of 1 % was observed in Poland (JUNKUSZEWA et al., 2016), and 9 % in Belgium (MICHIELS, et al., 2018) and Switzerland (SCHALLER et al., 2000). Seroprevalence from 10.05 % (AMEEN and KARAPINAR, 2018) to 15.3 % (AZKUR et al., 2011) was observed in Turkey, while in Spain it ranged from 25 % (LAGO et al., 2012) to 52.8 % (PEREZ at al, 2010) infected sheep. A much higher number of infected animals than in our study was found in Kosovo (34.8 %) (CANA et al., 2020) as well as in Greece (41.96 %) (KARANIKOLAOU et al., 2005). ALVES et al. (2017) found 6.72 %; positive sheep in Brazil. In China the MVV infection rate ranged from 4.60 to 50.00 % (ZHANG et al., 2013). According to BEHNAZ et al (2015) 34.5 % (CI 95 % 28.3 to 40.7 %) sheep and 89.6 %; (CI 95 % 74.4 to 98.8%) flocks were infected. in Iran.

From the comparison of seroprevalence in the counties included in this study, significant differences were found. The highest number of positive animals was found in Istria County (19.02 %, CI 95 % 13.35 –24.70 %) where the prevalence rate was 17.5 (CI 95 % 4.55 - 67.34), 3.5 (CI 95 % 1.89 – 6.50) times higher than in Primorje Gorski Kotar County and Lika-Senj County, respectively (P<0.01) (Table 1). The difference in the prevalence rate between the sheep populations in Lika-Senj County and Primorje-Gorski Kotar County was not significant (P = 0.086). However, some research studies show that the seroprevalence also varies between and within different geographical locations. VIDIĆ et al (2017) also reported different seroprevalence rates between counties in south Croatia, ranging from 9.6 % (Zadar County) to 21.5 % (Šibenik-Knin County).

Out of the total of 460 sheep examined, breed was specified in 451, of which 385 sheep belonged to indigenous breeds (Istrian Pramenka, Pramenka, Krk sheep, Pag sheep and Lika sheep) and 66 to non-origin domestic sheep (Jezersko-Solcava sheep, Romanov sheep and East Friesian sheep) (Table 2). The most infected breeds were three indigenous

breeds: Istrian Pramenka (22.97 %, CI 95 % 13.39 – 32.55 %) Pramenka (13.16 %, CI 95 % 2.41 – 23.91 %) and Lika sheep (9.78 %, CI 95 % 3.71-15.85 %), and the non-indigenous breed Jezersko-Solcava sheep (23.53 %, CI 95 % 11.89 – 35.17 %). The differences in seroprevalence between these breeds were not statistically significant (P>0.05) (Table 2). The lowest seroprevalence was found in two indigenous breeds: Pag sheep (1.04 %, CI 95 % - 0.98 – 3.07 %) and Krk sheep (1,18 %, CI 95 % - 1.12 – 3.48 %). Significant differences were observed between Istrian Pramenka and Lika sheep, Krk sheep and Pag sheep, with odds ratios of 2.78 (CI 95 % 1.17-6.47), 25.06 (CI 95 % 5.80 – 108.27) and 28.33 (CI 95 % 3.67 –218.64), respectively (P<0.05). Breed susceptibility to MVV infection and significant differences between breeds were also observed by CUTLIP et al. (1988) and BARQUERO et al. (2013).

No significant difference in seroprevalence between female (10.32 %, C I 95 % 7.47 - 13.17 %) and male (7.14 %, CI 95 % -6.34 - 20.63 %) sheep was found (prevalence ratio 1.45, CI 95 % 0.22 - 9.37) (P<0.05) (Table 2). No significant difference in seroprevalence between males and females was also reported by ARSENAULT et al. (2003), BEHNAZ et al. (2015) and ALVES et al. (2017). However, in the study conducted in the Dalmatian region by VIDIĆ et al. (2017) many more seropositive rams were found (19.96 %) than ewes (4.4 %). PREZIUSO et al. (2010) found than ewes were 3 times more likely to be positive than rams.

Out of 460 examined sheep, the age was specified for 396 sheep. Therefore, the age structure was examined to find out whether seroprevalence is related to age, since it is known that MVV infection is usually found in older individuals (PREZIUSO et al., 2010.). The mean age was found to be statistically significantly different between breeds as well as between gender (Tables 3 and 4). The mean age (in years) was higher in Pag sheep (7.26 ± 3.49), Krk sheep (6.41 ± 4.44) and Pramenka (6.70 ± 3.49), as well as Jezersko-Solcava sheep (7.67 ± 4.04) in comparison to Istrian Pramenka (5.50 ± 3.60) and Lika Pramenka sheep (5.26 ± 3.29). In Istrian Pramenka, where a higher seroprevalence was found, the average age was lower than in breeds with lower seroprevalence (Pag and Krk sheep). However, in Pramenka and Jezersko-Solcava sheep, a higher average age and seroprevalence were found. Although significant differences in age between breeds and gender, as well as between seropositive and seronegative animals were found (Table 2), no statistical significance in relation to seroprevalence was found (P<0.05) (Table 5). In the study performed by PREZIUSO et al. (2010) older sheep were 5 times more likely to be positive than younger animals.

According to the risk assessment, an association between prevalence rate and geographical location, as well as breeds, was confirmed (P<0.05) (Table 7). In Istria County it was about 21.38 (CI 95 % 2.88 – 158.71) and 1.98 (CI 95 % 1.95 – 8.49) more likely for sheep to be positive than in Primorje-Gorski Kotar and Lika-Senj County, respectively, (P<0.01). BARQUERO et al. (2013) also confirmed that the main risk factors associated with MVV infection in Spain were geographical location and breed, as well as the production system. Different seroprevalence rates according to region were also reported by BOJAR et al. (2018) in Poland, where the rate of positive flocks ranged from 27.03 % to 71.43 %.

Again according to the risk assessment, no association between prevalence rate and age, or prevalence rate and gender was found (Table 7). Our results are in accordance with CANA et al. (2020) who found no statistically significant differences in seroprevalence in sheep by age. On the contrary, BEHNAZ et al. (2015) found a relationship between seropositivity and age.

Since different breeds are bred in different geographical locations and possibly kept in different production systems, it is sometimes difficult to determine how much the MVV seroprevalence was related to breed and how much to the geographical location (county). In order to understand better the influence of breed as a risk factor, it was necessary to consider the examined breeds according to geographical location (Table 6 and 7). In Istria County, out of 184 examined sheep, most sheep belonged to the Jezersko-Solcava (N=74), Istrian Pramenka (N = 51) and Pramenka (N = 38) breeds,

with seroprevalence of 23.53 % (CI 95 % 11.89 - 35.17 %), 22.97 % (CI 95 % 13.39 - 32.55 %) and 13.16 % (CI 95 % 2.45 - 23.91 %), respectively. The results obtained in Istria County confirm that Jezersko-Solcava sheep are twice as likely to be positive than Pramenka sheep (OR = 2.03, CI 95 % 0.65 - 6.36) (P<0.01). In Primorje-Gorski Kotar County, the breed most examined was Krk sheep, among which a very low seroprevalence (1.18 %, CI 05 % -1.07 – 3.31) was found. In this region no significant difference was found in the probability of MVV infection between Krk sheep and Pag sheep (OR = 0.16, CI 95% 0.001 - 4.50, P=0.2815) Low seroprevalence was also found in Pag sheep in Lika-Senj County (1.09 %, CI 95 % -1.03 – 3.21 %). In this county significantly higher seroprevalence was found in Lika sheep (9.78 %, CI 95 % 3.71 -15.85 %) than in Pag sheep (1.09 %, CI -1.03 – 3.21 %) (P<0.01). This means that Lika sheep are about 10 times more likely to be positive than Pag sheep (OR=9.87, CI 95 % 1.22 - 79.56) in Lika –Senj County (P<0.05). The only breed that was examined in two geographical locations (Primorje-Gorski Kotar and Lika-Senj County) was Pag sheep, and no significant difference was found between Pag sheep in these two locations (Table 7).

Since there are no treatments or efficacious vaccines (REINA et al., 2009), serological diagnostic tests, such as enzyme-linked immunosorbent assays (ELISAs), are internationally prescribed and widely used (REINA et al., 2009; OIE 2018) for serological diagnosis of MVV infection. ELISAs have contributed to the reduction of MVV infection. They are most suitable for screening a large number of serum samples and are thus recommended for the control programs that should be applied, depending on prevalence rate and risk factors. For accreditation, the last step of a control programme should be applied when the seroprevalence is < 10 % (REINA et al., 2009). In our study the seroprevalence was found to be very low (1–9 %) and low (10–39 %), which is a promising factor in the implementation of control programs and eradication of the disease.

In conclusion, this cross-sectional study represents the first research into the risk factors associated with MVV in Croatia, as well as research into the seroepidemiology of MVV infection in sheep in southwestern Croatia. The results obtained in the present study confirm the circulation of MVV among sheep in the examined area. Detection of disease prevalence as well as understanding the risk factors are essential prerequisites for the application of control programs. Therefore, these results can contribute to a better understanding of MVV virus in Croatia, and help in the implementation of further epidemiological research and surveillance of MVV infection, as well as control programs, depending on the prevalence rate and risk factors.

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SAŽETAK

Cilj istraživanja bio je otkriti prisutnost infekcija virusom maedi visna (MVV) kod ovaca u tri županije u jugozapadnoj Hrvatskoj - Istarskoj, Primorsko-goranskoj i Ličko-senjskoj te procijeniti utjecaj geografske lokacije, pasmine, dobi i spola kao čimbenike rizika za stopu prevalencije. Ukupno je pregledano 460 nasumično odabranih ovaca postupkom ELISE ID Screen® MVV/CAEV indirektnog testa VISNAS-5P za otkrivanje protutijela protiv virusa maedi-visna. Stopa prevalencije za cijelu populaciju iznosila je 10,00% (CI 95% 7,26 - 12,74%). Najveća seroprevalencija MVV utvrđena je u Istarskoj županiji (19,02%, CI 95% 13,35–24,70%), zatim u Ličko-senjskoj županija (5,43%, CI 95% 2,17-8,70) i Primorsko-goranska županija (1,07%, CI 95% -1,03 - 3,17) (P<0,01). Usporedbom seroprevalencije između pasmina uočene su statistički znakovite razlike. Najveći broj pozitivnih ovaca nađen je kod istarske ovce (22,97%, CI 95% 13,39 - 32,55%), zatim jezersko-solčavske ovce (23,53%, CI 95% 11,89 - 35,17 %%), pramenke (13,16% , CI 95% 2,41 - 23,91%) i ličke pramenke (9,78%, CI 95% 3,71 - 15,85%). Stopa prevalencije u ostalih istraženih pasmina (krčka ovca, paška ovca i romanovska ovca) bila je niža od 2% (P<0,01). Statistički znakovite razlike u seroprevalenciji prema spola i dobi nisu ustanovljene. Kao glavni čimbenici rizika utvrđeni su zemljopisni položaj i pasmina. U Istarskoj županiji seroprevalencija je bila 4,77 (CI 95% 2,67 - 8,54) puta veća nego u ostalim istraženim područjima (OR = 5,66, CI 95% 2,97-10,79) (P<0,01). Uočene su znakovite razlike između jezersko-solčavske ovce i ličke pramenke (OR = 2,84, CI 95% 1,13-7,14) te istarske ovce u odnosu na pašku ovcu (OR 22,51 , CI 95% 16,99-119,73), krčku ovcu (OR 19,53, CI 95% 5,80-108,27) i ličku pramenku (OR 2,35, CI 95% 1,17-6,47). Rad predstavlja prvo istraživanje seroprevalencije i rizičnih čimbenika za MVV ovaca u jugozapadnoj Hrvatskoj. Rezultati ukazuje na prisutnost MVV infekcije te na važnost daljnjeg istraživanja bolesti u Hrvatskoj.

Ključne riječi: maedi-visna virus; ovce; seroprevalencija; čimbenici rizik